(Reprinted from Nature, Vol. 203, No. 4942, pp. 312–313, July 18, 1964)

Specific Precocious Protective Action of Toxoids

THERE have been several reports of a specific precocious protective action, not involving antibody response, of tetanus toxoid against tetanus toxin[1–8]. Massive doses of tetanus toxoid injected into mice protected them against a few lethal doses of tetanus toxin injected at the same time, or shortly before, or shortly after. Diphtheria toxoid, given in doses comparable on a basis of antibody-combining power units, had no such protective effect against tetanus toxin[5,6]. Suggestions have been made that the protective action of tetanus toxoid may be connected with its ability to prevent the fixation of tetanus toxin by nervous tissue[7–9] and ganglioside[10].

We have tested whether other toxoids have a similar protective action against their homologous toxins. A number of toxins were titrated by injecting 0·5 ml. volumes of serial two-fold dilutions into control and toxoid-treated animals. Toxoid and toxin were injected in immediate succession intramuscularly in the same hind-limb. In the case of tetanus, toxoid and toxin were also injected intraperitoneally and intravenously. The toxins and toxoids (in crude form) were kindly supplied by Dr. R. O. Thomson of the Wellcome Research Laboratories. In each case, the largest practicable dose of toxoid was used, which was 0·5 ml. of the solutions as provided by Dr. Thomson. Mice were used in all the experiments except those with diphtheria toxoid and toxin, where guinea pigs were used. The number of animals used for each dilution of toxin are shown in Table 1. Deaths were recorded for 7 days after the injection of toxin. All the animals that died did so within two days, except those injected with tetanus toxin, which died on all the days between the first and seventh day after injection.

The results of these experiments are shown in Table 1. The values given in column 9 were obtained by dividing the values in column 3 by the means of those in column 8; they are based on the assumptions that the toxin preparations used for making toxoid contained no toxoid before toxoiding and that homologous toxins and toxoids have the same molecular weights. *Cl. oedematiens* and diphtheria toxoids in the doses used had no protective effect; *Cl. welchii* Type *A* toxoid in the comparatively small dose used had a very doubtful protective effect; *Cl. welchii* Types *B*, *C* and *D* toxoids appear to have had specific protective effects greater than that of tetanus toxoid by several orders of magnitude. Column 5 shows

Table 1

1	2	3	4	5	6	7	8	9
Toxin and toxoid	Toxoid values per dose before toxoiding		No. of animals per dilution of toxin	Total No. of animals protected in titration	LD_{50}/ml. toxin		$L\mathcal{D}_{50}$ against which protection achieved $(x-y)/y$	Molecules toxoid protecting against one molecule toxin (mean)
	$L+$	LD_{50}			Control animals	Toxoid-treated animals		
					x	y		
Cl. welchii Type *A*	3·25	81	2 2	1 0	96 48	64 64	0·5 −0·25	?
Cl. welchii Type *B*	90	900	2 2	1 1	96 64	64 48	0·5 0·25	$2{\cdot}4 \times 10^3$
Cl. welchii Type *C*	120	$2{\cdot}4 \times 10^3$	2 2	2 2	96 96	48 48	1·0 1·0	$2{\cdot}4 \times 10^4$
Cl. welchii Type *D*	155	$3{\cdot}1 \times 10^4$	2 2 2	3 2 4	320 240 320	120 120 80	1·6 1·0 3·0	$1{\cdot}7 \times 10^4$
Cl. oedematiens	6·5	$4{\cdot}4 \times 10^3$	2	0	24	32	−0·25	$> 4{\cdot}4 \times 10^3$
Diphtheria	100	$3{\cdot}5 \times 10^3$	2	0	24	24	0	$> 3{\cdot}5 \times 10^3$
Tetanus, *IP*	600	$2{\cdot}7 \times 10^7$	6 6	0 3	16 8	8 6	1·0 0·33	$4{\cdot}0 \times 10^7$
IV	600	$2{\cdot}7 \times 10^7$	2	2	12	6	1·0	$2{\cdot}7 \times 10^7$
IM	600	$2{\cdot}7 \times 10^7$	2	3	12	4	2·0	$1{\cdot}4 \times 10^7$

that in some cases these conclusions are based on the survival of very few animals, and the values in column 9 must be regarded as qualitative rather than quantitative; nevertheless, we believe they support the conclusion that the specific precocious protective action of tetanus toxoid is not unique. Additional experiments showed that *Cl. welchii* Types *C* and *D* and diphtheria toxoids in the doses shown in Table 1 failed to protect against *Cl. welchii* Types *D* and *C* and tetanus toxins respectively.

This work was carried out under contract with S. Office of Naval Research (task 103–474).

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Printed in Great Britain by Fisher, Knight & Co., Ltd., St. Albans.